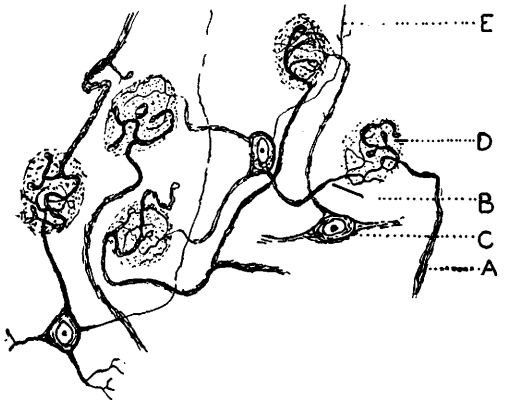

FIG. 3.
Outline drawing of microscopic anatomy of granule cell and granule cell glomerulus (modified from Cajal). A, Afferent fiber terminating as mossy fiber in protoplasmic glomerulus, D. C, Granule cell body. B, Dendrite of granule cell synapsing with afferent fiber in granule cell glomerulus. E, Granule cell axon.

cellular elements of the cerebellar cortex. We were not able to accomplish this technical procedure.

The Purkinje cell is not the only potential source of cellular elements for the maintenance of spontaneous electrical activity. A second possible source is the granule-basket cell combination in which the granule cell glomerulus deserves some comment. This non-nucleated protoplasmic structure located at the junction of the incoming mossy terminal with the dendritic terminals of the granule cells is approximately 15 μ in diameter. Fig. 3 illustrates the salient properties of the various parts of the granule cell. Note that the incoming afferent fiber (A) synapses within the glomerulus (D) on the way to the granule cell (C) dendrite (B). Note also that, except for a slightly granulated appearance, the protoplasm appears almost structureless.

To check the possibility of activity coming from the granule cell layer, we attempted to record directly from this layer with pick-up wire (26 gauge, insulated to tip). Although good activity could be recorded, and often better activity than that from the pial surface, which is approximately 250 μ from the granular layer, the answer is still equivocal because possible activity of nearby Purkinje cells may have spread to the needle tip or Purkinje cell axons passing nearby may have been conducting activity through the area to the underlying cerebellar nuclei. From this, one must conclude that if the intrinsic spontaneous electrical activity of the cerebellar cortex results from spontaneous activity of cellular aggregates, as seems likely, then the basket-granule cell Fig. 2 D,F (with glomerulus) combination appears to be a possible responsible agent. One thing is certain, either it is this cellular complex or it is the Purkinje cell itself which is responsible.

Summary. Evidence is presented to show: (a) that the fast electrical activity of the cerebellum is intrinsically maintained within this structure; (b) that it is not due to reverberating circuits through the cerebellar nuclei and back to the cerebellar cortex; (c) that it is probably due to the spontaneous activity of Purkinje cells and/or the granule-basket cell combination.

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Prevention of Secondary Infection due to *Pseudomonas aeruginosa* in Frostbitten Tissue. (17353)

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One of the major problems encountered in investigations on experimental frostbite is the prevention of infection of the frozen tissue. Without prophylactic treatment infection occurs in practically all cases of severe frostbite. The following report gives the results of the application of penicillin and sulfamylon*

* The sulfamylon hydrochloride powder was supplied by the Medical Research Department of Winthrop-Stearns, Inc.

TABLE I.
Incidence of Infection in Frostbitten Legs Treated Locally with Penicillin.

	Number	Not infected	Infected
Heparin treated	42	32	10
Non-heparin treated	36	16	20
Total	78	48	30

$\chi^2 = 6.82$ and $P = 0.008$.

heparin therapy and 128 did not. The occurrence of gangrene was practically the same in the two series. Of the entire group 48 saved the frostbitten leg completely, 42 lost part of the injured limb, 105 lost the leg completely, and 95 died, 14 of them after the final fate of the leg was decided.

Penicillin. Seventy-eight animals, heparinized and non-heparinized, received local treatment with penicillin ointment (1,000 units per

TABLE II.
Incidence of Gangrene in Infected and Non-infected Animals Treated with Penicillin.

	Number	Completely saved	Partially saved	Completely lost	Died
Non-infected	48	0	11	18 (2)*	19
Infected	30	0	4	22	4

* Number in parentheses indicates animals which died after the fate of the leg was decided.
$\chi^2 = 2.00$ and $P = 0.15$.

(p-aminomethylbenzenesulfonamide) to the injured area after severe experimental local cold injury.

The experiments were performed on 290 male white albino rabbits weighing 2,000 to 3,000 g. The injury was produced by immersing one depilated hind leg for 30 minutes in an alcohol bath cooled with dry ice to temperatures ranging from −10°C to −25°C. The exact experimental procedure is described in another paper.[12] After the experiment the frozen area was covered with wool fat and a sterile dressing applied. During the first week the dressings were changed every day, sometimes every other day. Infections usually became evident between the fourth and sixth day after injury. The infected frozen area appeared bluish green, swollen, and slightly translucent. Small hemorrhages in the parts bordering the healthy tissue were quite often present. Pus oozed from the injured area, especially from the region of the demarcation line. From the bluish discoloration of the bandages and from the characteristic odor it was assumed that the infection was caused by *Bacillus pyocyaneus.* The bacteriological examination of 2 cases revealed pure cultures of *Pseudomonas aeruginosa (B. pyocyaneus).* The clinical appearance was characteristic enough that no further bacteriological investigation was considered necessary.

Of 290 animals 162 received intravenous cc). In this group 30 animals showed signs of infection in the frostbitten area and 48 did not.

The degree of injury was the same in both groups of animals. The difference in the incidence of infection in the heparinized and non-heparinized animals is significant ($\chi^2 = 6.82$ and $P = 0.008$). We do not have an explanation for this finding. We do not believe the evidence sufficient to support the conclusion that heparin is effective in preventing local infection of frostbitten tissue. The incidence of gangrene of the frostbitten legs in the infected and non-infected animals shows no essential differences (Table II). The higher death rate in the non-infected group is mainly due to the fact that most of the deaths occurred before the fourth or fifth day when the infections usually became evident. The relation of partially lost to completely lost legs is slightly less favorable for the infected animals ($\chi^2 = 2.00$ and $P = 0.15$). But even if this difference were significant it would not necessarily mean that it was due to the infection. According to our observations the occurrence of infection of frostbitten tissue in rabbits is related to the diarrhea that develops in some animals after cold injury. There seems to be a higher probability for the appearance of diarrhea with increasing severity of cold injury.

Sulfamylon. One hundred and eighty ani-

TABLE III.
Comparison of the Effect of Local Treatment with Penicillin and with Sulfamylon.

	Number	Not infected	Infected
Penicillin treated	78	48	30
Sulfamylon treated	212	208	4
Total	290	256	34

$\chi^2 = 70.2$ and $P = <0.0001$.

mals were treated in exactly the same manner as the preceding group, except that instead of penicillin ointment an ointment with 3% sulfamylon was used. Of these animals 60 did not receive any other treatment while 120 were heparinized. Signs of local infection did not occur in these animals.

Another group of 32 rabbits was frostbitten in the same manner as in the preceding series. They were used for temperature measurements during freezing and thawing and received little care thereafter. They were routinely treated by local application of sulfamylon ointment, but the dressings were usually changed every second to fourth day. In this group 4 animals developed local infection at the site of injury in spite of the application of sulfamylon. Remarkably enough these infections were found after the dressings had not been checked for 3 days.

Even if we include the separate group of 32 animals with inadequate treatment, in which the only infections during sulfamylon treatment occurred, the results show undoubtedly the superiority of sulfamylon.

The statistical computation of these results shows that the difference is highly significant ($\chi^2 = 70.2$ and $P = <0.0001$).

Discussion. Since the introduction of p-aminomethylbenzenesulfonamide (marfanil or sulfamylon) it has been shown to be a very effective drug in the prevention and cure of local infection. It is effective against a most diversified number of organisms in clinical as well as laboratory tests.[1,3,5-10,13] Its effectiveness against *Pseudomonas aeruginosa (B. pyocyaneus)* which caused the infections in our frostbite experiments is of special interest. Clinical infections with *Pseudomonas aeruginosa* respond well to treatment with p-aminomethylbenzenesulfonamide.[2,5,6,9] The same results were obtained in bacterial culture tests.[6,8] We agree with Howes[6] that this drug is distinctly superior to penicillin in its effectiveness against *Pseudomonas aeruginosa.* But since the effect is bacteriostatic,[6,8] it seems necessary to continue the treatment regularly until the possibility of infection has passed. The usefulness of p-aminomethylbenzenesulfonamide in the prevention and cure of infection in frostbite has already been reported in former papers.[11,14] It is also effective in the treatment of deep burns[4] where similar tissue conditions exist.

Summary and conclusions. 1. Of 78 animals with severe experimental frostbite 30 cases of infection with *Pseudomonas aeruginosa (B. pyocyaneus)* occurred, although the frozen area was treated locally with penicillin ointment.

2. Of 212 animals with the same degree of cold injury and treated with 3% sulfamylon ointment, only 4 animals developed local infection with *Pseudomonas aeruginosa.* In 180 of these animals the dressings were changed daily and none became infected. The dressings of 32 animals were changed every 2 to 4 days; in this group 4 infections occurred.

3. Sulfamylon is superior to penicillin in its effect on *Pseudomonas aeruginosa* infections.

4. It is possible to control the infection due

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to *Pseudomonas aeruginosa* in experimental frostbite by daily local application of sulfamylon.

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Use of Heparin in Treatment of Experimental Frostbite. (17354)

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During World War II casualties from local cold injury were numerous. In some units they exceeded the number of casualties from every other cause. This was especially true of Air Force personnel on high altitude bombing missions. It was therefore quite natural that much interest was focused on the investigation of the mechanism and therapeusis of cold injury.

Among the publications on the treatment of local frostbite, the reports of Lange and his collaborators were the most remarkable.[1-5] These authors described the results of heparin in the prevention of gangrene in animals and human volunteers after local cold injury. The control cases in their experiments always developed extensive or complete necrosis of the frostbitten area whereas the heparin treated cases almost always escaped gangrene. The details of the experimental procedures and of heparin administration as used in their investigations will be discussed later.

Publications subsequent to those of Lange *et al.* did not confirm their results.[6,7,8] Because of the importance of the subject to the Air Force we deemed it advisable to repeat these experiments.

Material and Method. With few exceptions in the early series we used white male albino rabbits of more than 2,000 g body weight. Female animals were excluded since in one series they seemed to be more susceptible than males to internal hemorrhages after heparin administration. The animals were kept in the open air throughout the year, but at least one week before the experiment they were brought into the air conditioned animal house and kept at 25°C (77°F). The food consisted of Purina rabbit pellets and occasionally carrots; water was unlimited. In the course of the investigation during which more than 300 animals passed through our animal room only 2 animals died spontaneously.

During the first part of the experiment we followed the procedure of Lange *et al.*[2,5] as closely as possible, except that the animals were not anesthetized during freezing. The right hind leg was clipped and depilated with Zip Depilatory Cream from the ankle upwards and loosely covered with a rubber condom boot to about 2 cm to 3 cm above the knee joint. The prepared hind leg was immersed to the knee joint in a bath at −25°C (−13°F) for 30 minutes. The freezing bath consisted of 95% alcohol cooled with dry ice. After some experience it was possible to keep the bath within 0.5°C of the desired temperature. Immediately after freezing, the legs were

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